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Genetic Analysis of Cultured and Wild Populations of *Mytilus coruscus* Based on Mitochondrial DNA

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Abstract: DNA sequences from the mitochondrial gene cytochrome oxidase subunit I (mtDNA *COI*) were used to estimate the genetic variability in two wild populations and two cultured populations of the hard shelled mussel, *Mytilus coruscus*. Thirty haplotypes were identified in the four populations. The cultured populations exhibited a lower number of haplotypes and genetic diversity than those of the wild populations, suggesting that a small number of effective founding breeders contributed to the genetic variation of the cultured populations. No significant differentiation was observed between the cultured population and local wild population, implying that persistent gene flow occurred in these populations. This genetic survey is intended as a baseline for future genetic monitoring of *M. coruscus* aquaculture stocks.

Key words: *Mytilus coruscus*; Population differentiation; Genetic diversity; mtDNA *COI* gene

厚壳贻贝养殖群体与野生群体线粒体 DNA 的遗传分析

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摘要：采用线粒体 DNA *COI* 基因序列对厚壳贻贝 2 个养殖群体与 2 个野生群体的遗传多样性进行了研究。4 个群体共获得 30 个单倍型。结果显示：在养殖群体中单倍型的数量和遗传多样性要比野生群体的低，可能是由于只有少量的有效父母本对养殖群体的遗传变异有贡献所致。养殖群体与当地野生群体之间也未发生显著的遗传分化，可能是因为它们之间存在基因流。在今后厚壳贻贝养殖过程中，本研究可以用在对养殖群体进行遗传监测，从而保证养殖群体的遗传多样性水平。

关键词：厚壳贻贝；群体分化；遗传多样性；线粒体 DNA *COI* 基因
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The hard shelled mussel, *Mytilus coruscus*, is an economically important mussel which is widely distributed from coast of China, Japan, and Korea (Wang, 1997). It is documented that this species occurred from Dalian to Xiamen in China. The culture of *M.coruscus* has been carried out in only a few regions of China in the past decades. The cultured juveniles mainly originated from the collection of natural populations. In recent years, natural juveniles have decreased while mussel farmers have increased. Due to overexploitation, most mussel stocks have dramatically declined. Therefore, it is very important to obtain juveniles that are produced by artificial breeding to increase the juvenile population. The first hatchery stock is being developed in Zhejiang province, China. However, differences in allele frequencies between farmed strains and the wild source populations have been shown to be a result of breeding related individuals or the use of small numbers of individuals as brood-stock, leading to lower genetic variability of the farmed strains (McGinnity et al, 2003). Additionally, farmed strains of small population size are more sensitive to genetic drift and consequently have lower within population genetic diversity than wild populations (Allendorf, 1986). Genetic variability is the primary resource in the successful artificial propagation of any species. Proper management and breeding

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programs must be implemented to preserve genetic variability, and prevent inbreeding depression. However, for such programs to be successful, information on the genetic relationships among cultured and wild populations is essential.

Mitochondrial DNA has been used extensively for studies in molecular ecology (Avise, 2000). In comparison to allozyme or nuclear DNA, the higher mutation rate, smaller effective population size is expected to provide greater power to identify population structure. Additionally, mtDNA gives a better estimate of genetic differentiation than nuclear markers since it is approximately fourfold more sensitive to genetic drift and founder effects (Birky et al, 1983). Mitochondrial Cytochrome Oxidase I gene (mtCOI) has been used widely in marine species, such as *Tegillarca granosa* (Zheng et al, 2009)，due to adequate levels of variability and easy amplification via universal primers (Folmer et al, 1994).

In this study, we used the mitochondrial Cytochrome Oxidase I gene (mtCOI) to describe the genetic variability of two cultured, and two wild populations and also to quantify the genetic differentiation between them.

1 Materials and Methods

1.1 Samples

Specimens of *M.coruscus* obtained from both cultured and natural populations are listed in Tab. 1 and Fig. 1. The two wild populations were randomly sampled by divers from the subtidal zone of Shengsi and Lianjiang in 2007，respectively (coded WSS and WLJ). The two cultured populations were of Shengshan (coded CSS) and Huaniao (coded CHN), and also collected in 2007. The CSS population was collected from the cultured area of *M.coruscus*. In this area, the aquaculture practice had been carried out for three decades. A large number of the natural spat collected were commonly reared. The CHN population hatchery stock was the first-generation of offspring produced in spring 2005 using hundreds of wild caught *M.coruscus* in Shengsi (exact numbers not documented). Gill tissues were obtained and stored in 100% ethanol at room temperature until DNA extraction.

Tab. 1 Wild and cultivated mussels (*Mytilus coruscus*) obtained at different sites used in this study

Origin	Site	Abbreviation	Longitude	Latitude	Year
Wild	Shengsi	WSS	122°41′	30°51′	2007-11
Wild	Lianjiang	WLJ	119°53′	26°02′	2007-05
Cultured	Huaniao	CHN	122°40′	30°52′	2007-05
Cultured	Shengshan	CSS	122°48′	30°43′	2007-05

1.2 Extraction of DNA

Genomic DNA was isolated from gill using the standard proteinase K digestion and phenol/chloroform extraction procedures described by Shen et al (2006). DNA quality was assessed by running samples on 1% agarose gels, and DNA concentration was measured with an UV/visible spectrophotometer (Eppendorf AG 22331 Hamburg) for absorption at 260 nm. DNA was diluted to 50 ng/μL for polymerase chain reaction (PCR) amplifications.

1.3 Amplification of DNA and sequencing

PCR was carried out in 50μL reactions containing 100 ng DNA sample, 5 μL 10×PCR buffer, 2.0 μmol/L $MgCl_2$, 200 μmol/L dNTPs, 0.2 μmol/L primer, 1U Taq polymerase. A pair of universal primers was used in this study (forward primer LCO1490：5'-GGTCAACAA-ATCATAAAGATTGG-3'; reversal primer HCO2198：5'-TAAACTTCAGGGTGACCAAAAAATCA-3'(Folmer et al, 1994). PCR was performed using the Eppendorf PCR system programmed for an initial denaturation step of 3 min at 94℃ followed by 35 cycles, each consisting of 94℃ denaturation for 50 second, 48℃ annealing for 50 second and 72℃ extension for 1 min. A final extension of 10 min was performed at 72℃ and the PCR products were then held indefinitely at 4℃. A negative control, consisting of all the reaction components except template DNA, was also included for each of amplification.

PCR products were visualized using 1.5% agarose gel stained with ethidium bromide. All amplified products were purified using TIANquick Midi Purification Kit (Tiangen, China). Purified PCR products were directly sequenced in both directions using the PCR primers on an Applied Biosystems ABI 3730 DNA sequencer.

1.4 Data analysis

For all sequence analyses, sequences were aligned with BioEdit Sequence Alignment Editor version 7.0.9

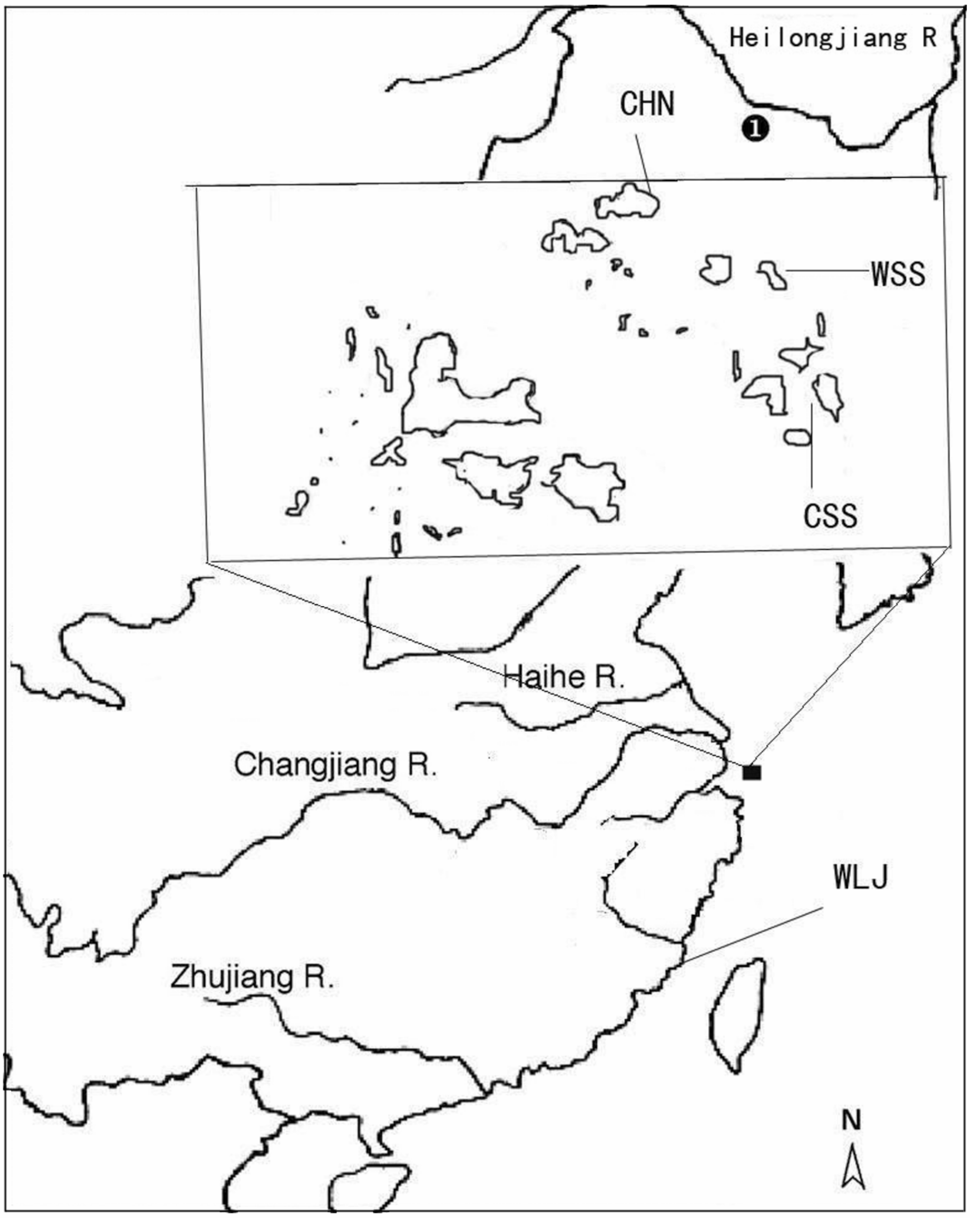


Fig. 1 Sample locations of two wild and two cultured *Mytilus corcuscus*

(Hall, 1999) and saved in the Fasta format. The identical haplotypes in the aligned matrix were identified and collapsed using COLLAPSE version 1.2 (Posada, 2006). Nucleotide composition was computed in MEGA version 4.0 (Tamura et al, 2007). Genetic diversity among wild and cultivated populations was estimated using haplotype number (*N*), gene diversity (*h*), and nucleotide diversity (π) as implemented in DnaSP version 4.0 (Rozas et al, 2003). Gene diversity was calculated according to Nei (1987), using the probability that two randomly chosen haplotypes of the sample are different. Nucleotide diversity was also computed according to Nei (1987), as the probability that two randomly chosen homologous sites are different. Genetic diversity was compared between farmed and wild populations of mussel using FSTAT (Goudet, 1995).

Pairwise estimates of F_{st} and corrected average pairwise population distances among all populations were obtained using the program Arlequin3.1 (Excoffier et al, 2005). The significance of these estimates was tested by comparing observed F_{st} with a null distribution obtained by 10 000 random permutations of the data set (Excoffier et al, 1992).

2 Results

2.1 Characteristics of mtDNA COI of *M.coruscus*

Once all sequences were aligned, a total segment length of 573 bp of cytochrome oxidase subunit I (*COI*) was obtained. The average nucleotide composition in our samples is A=33.6%, C=23.9%, G=15.8%, T=26.6%, with an average G+C content of 39.7%. These sequences include 59 variable sites, of which 41 are singleton variable sites and 18 are parsimony informative sites. Sequences have been deposited in GenBank under the following accession number range: FJ495257-FJ495286.

2.2 Genetic variability and population structure within populations

The samples examined show a wide range of values for the number of haplotypes, gene diversities and nucleotide diversities (Tab. 2). A total of 30 haplotypes were obtained for wild and cultured populations of *M. coruscus* (Tab. 3). The number of individuals of different haplotypes ranged from 1 to 18. The haplotype Hap2 had the greatest number of individuals across all samples. The wild WSS population had the greatest number of haplotypes, whereas the cultured CHN population originating from the Shengsi area had the fewest number of haplotypes. Every population had population-specific haplotypes. In the cultured WLJ population, only 3 specific haplotypes were found, exhibiting a decrease of specific haplotypes. In contrast, the samples from the wild WSS population exhibited the greatest number of population-specific haplotypes. The samples from natural populations exhibited the greatest number of haplotypes and population-specific haplotypes compared to cultured samples.

Tab. 2 Genetic diversity parameters of *Mytilus coruscus* populations examined

Population	Number of individuals(n)	Number of haplotypes(N)	Gene diversity(h)	Nucleotide diversity(π)
WSS	20	13	0.911	0.00898
CHN	13	6	0.718	0.00293
CSS	20	12	0.911	0.00834
WLJ	13	10	0.923	0.00948
Overall	66	30	0.895	0.00832

The samples from wild WSS population had an H value of 0.911 and π of 0.00898, compared to the aquaculture samples CHN and CSS with H values of 0.718, 0.911 and π values 0.00293, 0.00834, respectively. The wild WSS population had 3-fold higher nucleotide diversity than the cultured CHN population obtained through artificial propagation. All cultured populations exhibited the less genetic diversity for gene diversity and nucleotide diversity compared to the wild populations.

2.3 Genetic differentiation among populations

Tab. 4 shows different indices of dissimilarity between pairs of populations, including genetic distance and F_{st} statistics. When populations were compared pairwise, the highest F_{st} values appeared in CHN/WLJ pairs (F_{st}=0.2991, P<0.01). The F_{st} values for WLJ/WSS were also statistically significant (F_{st}=0.1414, P<0.05). The comparison of populations WSS and CHN, CSS show low F_{st} values, indicating that these two populations were not highly divergent. But interestingly, genetic differentiation between CHN and CSS was significant (F_{st}=0.1376, P<0.05). In concordance with F_{st} values, the pairwise Nei's genetic distance value was low between WSS and CSS, CHN populations (0.099 and 0.298, respectively). The largest genetic distance was between WLJ and CHN (1.735).

3 Discussion

The artificial breeding program for this species starts for a short time and will expand accordingly. There is no previous genetic record of this species and no attempt has been made to assess the genetic status of both wild and cultured populations of this species. This is the first study to demonstrate that a mitochondrial marker can be used to monitor changes in the genetic diversity of the hard-shelled mussel (*M.corcuscus*) during domestication.

Genetic variability is an important attribute of species under domestication, since those with higher levels of variation are most likely to present high additive genetic variance for production traits (Alarcon et al, 2004). In this study, genetic analysis of mitochondrial COI gene sequences revealed higher genetic variability for gene diversity and nucleotide diversity both in wild and cultured *M. corcuscus* than for the same sequence regions in other marine organism. Results from mitochondrial COI gene sequences in crayfish showed

Tab. 3 mtDNA haplotypes distribution of *Mytilus coruscus* populations (GenBank accession no and number of individuals)

Name	GenBank accession No	WSS	CHN	CSS	WLJ	Total
Hap1	FJ495257	1				1
Hap2	FJ495258	6	7	4	1	18
Hap3	FJ495259	2	1		1	4
Hap4	FJ495260	1	2	1		4
Hap5	FJ495261	1				1
Hap6	FJ495262	1				1
Hap7	FJ495263	1				1
Hap8	FJ495264	2		5	4	11
Hap9	FJ495265	1				1
Hap10	FJ495266	1				1
Hap11	FJ495267	1				1
Hap12	FJ495268	1				1
Hap13	FJ495269	1				1
Hap14	FJ495270		1			1
Hap15	FJ495271		1	2	1	4
Hap16	FJ495272		1			1
Hap17	FJ495273				1	1
Hap18	FJ495274				1	1
Hap19	FJ495275				1	1
Hap20	FJ495276				1	1
Hap21	FJ495277				1	1
Hap22	FJ495278				1	1
Hap23	FJ495279			1		1
Hap24	FJ495280			1		1
Hap25	FJ495281			1		1
Hap26	FJ495282			1		1
Hap27	FJ495283			1		1
Hap28	FJ495284			1		1
Hap29	FJ495285			1		1
Hap30	FJ495286			1		1

Tab. 4 Pairwise estimates of Nei's genetic distance (above diagonal) and F_{st} (below diagonal) between *Mytilus coruscus* populations

	WSS	CHN	WLJ	CSS
WSS		0.099^{NS}	0.755^{*}	0.298^{NS}
CHN	0.0230^{NS}		1.735^{**}	0.954^{**}
WLJ	0.1414^{*}	0.2991^{**}		-0.050^{NS}
CSS	0.0466^{NS}	0.1376^{*}	-0.0087^{NS}	

* P<0.05; ** P<0.01; NS, not significant.

nucleotide diversity ranging from 0.01% to 0.43% (Trontelj et al, 2005). In the mud crab *Scylla serrata*, the COI sequence divergence ranges from 0.17 % to 0.46% and gene diversity ranges from 0.37 to 0.85 (Fratini & Vannini, 2002). Relatively high levels of DNA diversity characterized the population of the bivalve mollusc *Congeria kusceri* (haplotype diversity=0.66 in the COI gene) (Stepien et al, 2001). However, the mtDNA nucleotide and gene diversity were reduced in the cultured population. A slight decrease of genetic variability in cultured populations had been observed in many fish and mussel species (Lundrigan et al, 2005; Pampoulie et al, 2006; Wang, 2007; Kong & Li, 2007; Shu et al, 2008). The reduced genetic variability we observed in the farmed strains is probably due to a low number of successful breeders during the foundation period, similar to a recent bottleneck in terms of impact on genetic variability (Allendorf, 1986). The greatest reductions in number of haplotypes, nucleotide diversity and gene nucleotide observed in cultured CHN population in this study are probably caused by the use of small numbers of brood-stock collected from wild populations. The reductions in the cultured CSS population are probably contributed to collection of natural spat from small numbers of parents and samples examined. A more precise assessment of the genetic variability in cultured populations can be made, were the magnitude of the genetic variation of wild populations

made available. This is almost a requirement when the number of generations of the cultured populations is small and there has not been enough time for the variation to be reduced to a detectable level.

The comparison of the F_{st} values (Tab. 4) between CSS, CHN and WSS populations showed that the values were not significantly different. This indicates that no significant genetic differentiation was detected between CHN, CSS and WSS populations. Similar findings were reported for fish (Yang et al, 2008), marine mussel (Jiang et al, 2007; He et al, 2008). The slow genetic differentiation might be due to persistent gene flow caused by cultured juvenile practice in open sea, close to the wild population. But significant genetic differentiation observed between WLJ and WSS, CHN populations, was probably associated with geographic factors. This regional variation could be an important source of diversity for both genotypic and phenotypic traits to be selected in accordance with aquaculture goals (Lundrigan et al, 2005).

Large scale approaches to assess the genetic structure of wild and cultured populations, such as those reported here for *M. coruscus*, are most suitable to provide insights about the evolutionary history of a population and its potential as a source of variation for cultured stock. More detailed information obtained in future studies will be used as a baseline for genetic monitoring of *M.coruscus* aquaculture stains, in particular, the first-generation, recently cultured population. Through population management, inbreeding can be prevented through the maintenance of population size above a critical level, with regular observation of heterozygosity levels and breeding to maximize genetic variability and minimize inbreeding (Barroso et al, 2005). Future farming practice should avoid culturing in areas in which local wild population inhabit, since wild populations represent the primary source of genetic variability for aquaculture stocks. This genetic survey is intended as a baseline for future genetic monitoring of *M.coruscus* aquaculture stocks.

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上海海洋大学水产动物种质资源与创新团队简介

水产动物种质资源与创新团队是上海海洋大学水产养殖国家重点学科的骨干研究力量，也是省部共建水产种质资源发掘与利用教育部重点实验室的基本研究队伍。

上海海洋大学是国内最早开展水产动物种质资源与创新研究的单位，世界水产养殖终身成就奖获得者——李思发教授 80 年代初回国后创建了这个研究领域，至今已有近 30 年的历史，在国内外产生了广泛的学术影响。李思发教授领导的课题组历时 20 年经过 6 代系统选育，获得了生长速度比原种快 30%、体形好、遗传性状稳定的团头鲂新品种“浦江一号”，它是世界上第一个经选育的草食性鱼类新品种，也是上海市育成的第一个水产动物良种，该项目获 2002 年度上海市科技进步一等奖、2004 年度国家级科技进步二等奖。他领导的课题组还于 2006 年初选育出新吉富罗非鱼新品种，以该新品种为核心的“从吉富到新吉——尼罗罗非鱼种质创新与应用”项目荣获 2007 年度上海市科技进步一等奖。

该团队年轻学术带头人李家乐教授是李思发教授的学生，他领导的课题组从 1998 年开始，对我国具有悠久养殖历史、产量占世界产量 95%以上的淡水珍珠蚌，开展了系统的种质评价与筛选，获得了三角帆蚌鄱阳湖新品系，并率先将三角帆蚌和从日本引进的池蝶蚌进行杂交，获得了新品种“康乐蚌”。“淡水珍珠蚌新品种选育和养殖关键技术”项目获 2008 年度上海市科技进步一等奖。

目前，该团队拥有教师 16 名，其中教授 6 人，副教授 5 人，是一支以中青年和具有海外留学经历的教师为主的创新型团队。该团队拥有研究生 75 名，其中博士研究生 12 人。

最近五年来，该团队获得 3000 多万科研经费，其中重要的科研项目有：“973”前期研究专项、“863”、科技支撑、农业产业体系、国家自然科学基金重点等。获国家科技进步二等奖 1 项，上海市科技进步一等奖 3 项，省部级二等 2 项、三等奖 10 余项；在公开的学术刊物上共发表论文 253 篇，其中 SCI、EI 论文 26 篇；主编或参编专著 5 部、教材 5 部，其他编著 6 部；申请专利 12 项，其中获得 3 项。

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